overload by altering the equilibrium of the Na/Ca exchanger to impair forward-mode (Ca extrusion), and favor reverse-mode (Ca influx) exchange. In turn, this Ca overload is expected to further activate CaMKII thereby forming a pathologic positive feedback loop of ever-increasing CaMKII activity, [Na]i, and [Ca]i.

We developed a computational framework to interrogate this potentially arrhythmogenic positive feedback in the mouse ventricular myocyte in both control conditions and when CaMKIIδC is overexpressed as in transgenic mice.

In control conditions, simulation of an increase in [Na]i causes the expected increases in [Ca]i, CaMKII activity, and target phosphorylation, which degenerate into unstable Ca handling and and electrophysiology at high [Na]i gain. Notably, clamping CaMKII to basal levels ameliorates but does not completely offset this outcome, suggesting that the increase in [Ca]i per se plays an important role. The effect of this CaMKII-Na-Ca-CaMKII feedback is more striking in CaMKIIδC overexpression, where high but not low [Na]i causes delayed afterdepolarizations, which can be prevented by clamping CaMKII phosphorylation of L-type Ca channels, ryanodine receptors and phospholamban to basal levels. In this setting, Na loading fuels a vicious loop whereby increased CaMKII activation perturbs Ca and membrane potential homeostasis. High [Na]i is also required to produce instability when CaMKII is further activated by increased Ca loading due to β-adrenergic activation.

Our results support recent experimental findings of a synergistic interaction between perturbed Na fluxes and CaMKII, and suggest that pharmacological inhibition of intracellular Na loading can contribute to normalizing Ca and membrane potential dynamics in heart failure.

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Contribution of the Mechanical Loads to Susceptibility to Arrhythmia in Subendocardial and Subepicardial Ventricular Myocytes

Anastasia Vasilyeva[1,2], Olga Solovyova[1,2], Vladimir Semenovich Markhasin[1,2].
[1]Institute of Immunology and Physiology, Ekaterinburg, Russian Federation, [2]Ural Federal University, Ekaterinburg, Russian Federation.

Motivation: It is shown that repolarisation alternans is initiated in the endocardial (ENDO) regions rather than the epicardium (EPI) in the left ventricle (LV) of different animal species (Cordeiro et al, 2007). However, possible contribution of the mechanical factors to the development of rhythm disturbances in ENDO and EPI myocytes has not been sufficiently investigated.

Methods: We have shown recently that intracellular mechanisms of mechano-electric feedback contribute to regional differences in the electrical and mechanical activity between virtual ENDO and EPI ventricular cardiomyocytes of guinea pig (Vasilyeva et al, 2012). In this study we used the models to study effects of the mechanical conditions on vulnerability of EPI and ENDO myocytes to rhythm disturbances induced by an increased density of fast sodium current (I_{Na}).

Results: In isometric (heavy-loaded) mode of contractions, increased I_{Na} caused early afterdepolarizations (EAD) in ENDO, but not in EPI cells, along with action potential (AP) and contraction alteration, rapid fall down of the twitch force with subsequent force recovery in several beats.

In isotonic mode of contractions, increased I_{Na} induced the similar responses in ENDO cells as under isometric conditions independently of the afterload value. In EPI cells low-loaded mode of contractions induced spontaneous excitations, which completely stopped cell shortening ("sudden cardiac arrest").

Conclusion: The modeling results suggest that ENDO myocytes are more vulnerable to EAD at increased INa density than EPI cells in rather wide range of the mechanical loads. A decrease in the mechanical load may contribute to arrhythmia induction in both the EPI and ENDO cardiomyocytes. Supported by UB of the RAS (12-M-14-2009, 12-II-4-1067) and RBRF (14-01-31134, 13-04-00365).

3699-Pos Board B427

Intra-Myocardial Slow Force Response in Heterogeneous Myocardium

Olga Solovyova[1,2], Pavel Konovalov[1], Elena Lobova[1], Vladimir S. Markhasin[1,2].
[1]Institute of Immunology and Physiology, Ekaterinburg, Russian Federation, [2]Ural Federal University, Ekaterinburg, Russian Federation.

Recently we have reported on an intra-myocardial slow force response (SFR_IM) of myocardium to changes in the mechanical environment (Markhasin ea, PBMB, 2012). In contrast to SFRs referred to changes in cardiac muscle contractility in response to external (to the heart) mechanical stimuli, SFR_IM ensued from internal mechanical interactions of muscle segments in heterogeneous myocardium. We revealed SFR_IM in two interacting myocardial elements, referred to as the muscle duplex, presenting a simplest possible model of heterogeneous myocardium. We found beat-to-beat changes in the overall duplex force developed by end-to-end coupled muscles upon duplex formation. After duplex disconnection, the individual force produced by each muscle differed significantly from the baseline level prior to muscle coupling, thus pointing out to changes in muscle contractility. SFR_IM in muscle duplexes were accompanied with slow and opposite changes in the action potential and Ca^{2+} transient in the cardiomyocytes of interacting muscle elements. The SFR_IM phenomena were initially identified by means of mathematical modeling, and subsequently confirmed in physiological experiments involving native cardiac muscles. The SFR_IM were specified for isometric contractions of in-series duplexes where cyclic deformations of muscle segments under a constant length of the pair governed intracellular mechano-chemical and mechano-electrical mechanisms of excitation-contracting coupling contributing to the SFR_IM. Here we present effects of the mechanical load on the SFR_IM in isotonic and physiological modes of contraction of in-series muscle duplexes. We showed that muscle interaction during the isometric phase of duplex contraction determine the amplitude and duration of subsequent duplex shortening, depending on the load value. SFR_IM modifies the force-length and force-velocity dependences in interacting muscles depending on the duplex activation sequence. The results suggest the essential role of SFR_IM in the isovolumetric phase of ventricular contraction in the intact heart. Supported by UB RAS (12-M-14-2009, 12-II-4-1067).

3700-Pos Board B428

New Method for Determining the Total Calcium Content of Tissue Applied to Whole Skeletal Muscles from Mice with and Without Calsequestrin Knocked Out

Sandrine A. Kake-Guena[1], Cedric R.H. Lamboley[2], Patrice Bouchard[3], Jerome Frenette[3], Eric Rousseau[1], Robert T. Dirksen[4], Feliciano Protasi[5], Paul C. Pape[1].
[1]Physiologie et biophysique, Université de Sherbrooke, Sherbrooke, QC, Canada, [2]Institute of Sport, Exercise and Active Living, Victoria University, Melbourne, Australia, [3]Réadaption, Université de Québec - CRCHUL, Quebec City, QC, Canada, [4]Pharmacology and Physiology, University of Rochester Medical Center, Rochester, NY, USA, [5]Neuroscience/Imaging, Gabriele D'Annunzio University, Chieti, Italy.

The concentration of total Ca in whole fast-twitch skeletal muscle (EDL) from mice was determined using the Ca-dependent UV absorbance spectra of the Ca chelator BAPTA. Tissue was added to a solution containing 120 mM KCl, 2 mM HEPES, 0.15 mM BAPTA and 0.5% (w/v) SDS (sodium dodecyl sulfate) at pH 8.0 and homogenized, followed by addition of more solution to give ~10 mg of muscle mass per 1 ml of final solution and then centrifugation. BAPTA is expected to bind almost all of the Ca initially in the muscle. The concentration of total Ca in the muscle, denoted $[Ca_T]_M$ (concentration referred to whole tissue volume) was obtained with Beer's Law from the difference of absorbance of the supernatant and the absorbance with no Ca on BAPTA, obtained by addition of EGTA. $[Ca_T]_M$ increased approximately linearly with decreasing muscle weight, increasing from a best-fit value of 1.34 to 4.14 mM from, respectively, the highest to lowest muscle weights of 12.7 and 5.2 mg. This suggests that a smaller muscle might increase its Ca content in response to the work demand resulting in a larger specific force thereby compensating for its smaller mass. With only the skeletal muscle isoform of calsequestrin (CSQ1) and with both isoforms (CSQ1 and CSQ2) knocked out, the values of $[Ca_T]_M$ were about half the control-muscle values on average, taking into account the $[Ca_T]_M$ vs. muscle weight relationship above. Since almost all of the Ca in muscle is thought to be bound to calsequestrin in normal resting muscle, a much greater decrease in $[Ca_T]_M$ was expected. We hypothesize that a significant amount of Ca is bound to other sites in the CSQ knock-out muscles.

3701-Pos Board B429

Optogenetic Control of Skeletal Muscle Excitability

Marino Di Franco, Marbella Quiñonez, Julio Vergara.
Physiology, UCLA, Los Angeles, CA, USA.

We assessed the feasibility of using light-activated channels and pumps to modulate the excitability of adult skeletal muscle fibers. Fibers were enzymatically isolated from FDB muscles of the mouse and studied under current- or voltage-clamp conditions using a two-microelectrode amplifier. Fibers were kept in Tyrode at a resting (or holding) potential of −90mV. Contraction was arrested by adding 40mM EGTA to the pipettes' solution. Plasmids coding channelrhodopsin-2-EYFP [CHR2; Addgene] and archaerhodopsin-3-ECFP [ARCH; subcloned from pFCK-ARCH-CFP (Addgene) into pECFPN1], were transfected into FDB fibers via in vivo electroporation.